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EFFECT OF FOLIAR SPRAY OF CALCIUM CARBONATE AND ZINC SULPHATE ON FRUIT QUALITY OF KINNOW MANDARIN

Laila Zaman[1,*], Waqar Shafqat[1], Naseem Sharief[2], Kashif Raza[3], Safeer ud Din[1], Muhammad Ahsan Qureshi[1], Saeed Ahmad[1] and Muhammad Jafar Jiskani[1,*]

[1]Institute of Horticultural Sciences, University of Agriculture, Faisalabad-38040, Pakistan; [2]Horticultural Research Institute, Ayub Agricultural Research Institute, Faisalabad, Pakistan; [3]Date Palm Research Station, Jhang, Punjab, Pakistan
[*]Corresponding author's e-mail: nainashah5@@hotmail.com; jjaskani@uaf.edu.pk

Kinnow mandarin is most pronounced fruit being grown in Pakistan because of its excellent fresh intake, processing value, flavor, quality, high yield and better adaptability. Present study was conducted to study the effects of preharvest foliar spray of zinc sulphate and calcium carbonate to improve the fruit quality of Kinnow mandarin (*Citrus reticulata* Blanco). Fruit bearing trees of 12-15 years old were sprayed with calcium carbonate and zinc sulphate @ 3% and 4% concentration. Foliar application of $CaCO_3$ and $ZnSO_4$ significantly increased the fruit weight, fruit diameter, juice percentage, TSS, ascorbic acid, total antioxidant, total phenolic, carotenoids and flavonoids contents. $ZnSO_4$ @ 4% gave the best results for improvement of Kinnow fruit quality and yield.

Keywords: Rutaceae, Kinnow mandarin, fruit quality, micronutrients, preharvest spray, fruit drop, calcium pectate.

INTRODUCTION

Citrus belongs to family Rutaceae in plant kingdom. It is significant for production in large scale and worldwide distribution. Citrus fruits have significance due to their distinct taste and beneficial values. Juice is soothing, refreshing and delightful (Ahmed *et al*., 2007). Nutrients are usually quickly available to plants as foliar spray than soil application. Foliar spray of micronutrients is 7 to 21 times extra effective than applied in soil. $ZnSO_4$ plays important role in increasing the production by improving the fruit quality and reduce fruit drop. Foliar application of 4 to 12 kg/ha zinc ($ZnSO_4$) gave beneficial effect on chemical and physical attributes of fruits, but the lowest application of Zn indicates the better flavor and juice contents, total sugar and vitamin C (Altaf and Khan, 2008). While fruit weight, TSS, diameter, ascorbic acid and juice fraction are the best obtained at the rate of 0.06% Zn in the form of foliar spray on Kagzi lime (Ashraf *et al*., 2012). Basically, Zn acts as a metal activator for numerous enzymes, including RNA polymerase carbonic anhydrase, super oxidase dismutase and alcohol dehydrogenase. Zn deficiency limits RNA synthesis, which inhibits the synthesis of protein. It is involved in tryptophan biosynthesis and precursor of auxin IAA. Zn holds more significance besides imparting sustainability in production/productivity by reducing the fruit drop, and granulation. Zinc at the rate of 0.3-0.8% on 13 years old mango trees at bloom stage gave the fabulous enhancement in TSS, total sugars and fruit weight. Zinc effect on fruit quality and yield of mango, also increase TSS% in fruits and decrease the alternate bearing in Valencia orange and mandarins (Tariq *et al*., 2007).

Calcium plays significant role in sustaining fruits quality, preserve fruit firmness, proliferation of vitamin C, decrease breakdown of storage rotting and reduction in browning of apple. It also defends membrane disorganization and protects apparent-free space of tissue generally related with senescence and sustains the protein manufacturing capability of cell. Calcium increases the mechanical power of cell wall because it is the main component of cell wall as calcium pectate in plants which play significant role in establishment of pedicel attachment to proximal of fruit; thus, reduce fruit drop (Guardiola and Garcia, 2000). Commercially preharvest spray of calcium delays senescence and increases consumer acceptance with less damaging effect during fruit storage (Lester and Grusak, 2004). Smaller amounts of calcium carbonate have been effectively used to decrease softening in fresh fruits. High level of calcium contents in fruit may sustain membrane permeability and decrease the process of ripening during storage and it also increases fruit retention because it stimulates the development of lignin and cellulose and stimulate translocation and formation of carbohydrates (Aguayo *et al.*, 2008). The present study was initiated to investigate the preharvest foliar application of zinc sulphate and calcium carbonate to improve the fruit quality characteristics (physical and chemical) in Kinnow mandarin.

MATERIALS AND METHOD

The proposed study regarding the effects of foliar spray of calcium carbonate and zinc sulphate on the fruit quality of

Kinnow mandarin was initiated during December. Uniform mature fruits of Kinnow mandarin were harvested during February. Physicochemical analysis of fruits was performed in the Pomology Lab. There were five treatments including the control with three replications. The combinations of treatments were as T_0 = Control, T_1 = 3% $CaCO_3$, T_2 = 4% $CaCO_3$, T_3 = 3% $ZnSO_4$, T_4 = 4% $ZnSO_4$.

***Physical parameters*:** Fruit samples (15 fruits per sample) were weighed with the help of digital balance and average fruit weight was calculated by dividing total sample weight by number of fruits in sample. The diameter of twenty randomly selected fruits from each plant was measured at equator of each fruit with the help of Vernier caliper and average fruit diameter was calculated. Juice of each sample was extracted and sieved to eliminate pulp and then weight of each sample was obtained.

***Biochemical parameters*:** A digital refractometer ATAGO, RS-5000 (Atago, Japan) was used to measure TSS of fruit juice. The instrument was calibrated with distilled water before and during use. A drop of extracted juice sample was placed on clean prism of the instrument; reading was taken directly from refractometer and was expressed as °Brix at room temperature (24-26°C).

Ascorbic acid contents of juice were determined following the method described by Ruck (1961). 5 mL of sieved juice was taken in 100 mL volumetric flask and volume was made by adding 0.4% oxalic acid solution. Out of this, 5mL filtrated aliquot was taken and titrated against 2, 6-dichlorophenolindophenol dye to light pink color end point, persisted at least for 15 seconds.

For phenolic content, added 100 µL of juice sample in Eppendorf tubes and added 200µL 10% F.C. regents and vortexes thoroughly for few seconds. Then added 800µL Na_2CO_3 in each tube and again vortexed for few seconds and incubated the tubes at room temperature for 1 to 2 hours. Blank sample was also prepared by using 100µL of extraction mixture (M.A.A. & HCl) instead of sample. Transferred 200µL sample and blank to a clear 96 well microplate and read absorbance at 765 nm with spectrophotometer for phenolics.

For antioxidants, 50µL fruit juice was added with 5ml 0.004% (4mg/100ml) of methanol solution of DPPH and left for 30 minutes incubation at room temperature and measured absorbance at 517 nm. The same procedure was repeated with 50, 100 and 150µl extracts and average was calculated of all three extracts for antioxidants.

Total carotenoids were determined by the method described by Lalel *et al*. (2003). 1 gram of fruit pulp was ground with 0.05 gram of magnesium carbonate in silica sand by glass. Extraction was made twice by using acetone: n-hexane (75: 60, v/v) mixture (20 ml/sample). The sample were centrifuged at 12000 rpm. The final yellow color was appeared in tubes and extract was obtained in separating funnel and rinsed with the 40 ml of 10% NaCl and distilled water 20% with washing of both solutions. The samples were read in 436 nm absorption in spectrophotometer.

Colourimetric Aluminum Chloride method was used for flavonoid determination. Added 0.5 ml of each fruit extract separately with 1.5 ml of methanol, 0.1ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water and left at room temperature for 30 minutes. The absorbance of extraction mixture was measured at 415nm with a double beam Perkin Elmer UV/Visible spectrophotometer (USA). Total flavonoid contents were calculated as quercetin from a calibration curve and reading was expressed as TFC (mg of CEQ/100 g of FW).

***Experimental design*:** The experiment was designed according to Randomized Complete Block Design (RCBD) with four treatments and three replications. The data was analyzed by analysis of variance and treatment means were compared by applying DMR test.

RESULT AND DISCUSSION

***Fruit weight (g)*:** Application of zinc sulphate @ 4% (T_4) showed maximum fruit weight (182.87g). Results shown by T_1 ($CaCO_3$ @ 3%) 166.03g and T_3 ($ZnSO_4$ @ 3%) 165g were better than T_2 ($CaCO_3$ @ 4%) 152.13g (Fig. 1a) but non-significant among each other while untreated fruits exhibited minimum fruit weight (122.83g). Foliar application of Zn revealed the highest fruit weight in treated fruits as compared to untreated fruits in Kinnow mandarin (Mishra *et al.*, 2003). Another scientist Bhardwaj *et al.* (2010) concluded that calcium application had no noticeable improvement in average fruit weight of cherry.

***Juice percentage (%)*:** Out of all the treatments, maximum juice percentage was found in T_4 ($ZnSO_4$ @ 4%) 639.80% (Fig. 1b). The juice percentage of T_3 ($ZnSO_4$ @ 3%) and T_2 ($CaCO_3$ @ 4%) were better than T_1 ($CaCO_3$ @ 3%) but non-significant among each other (454.71%, 448.56% and 352.06 %, respectively) while untreated fruit gave minimum juice weight percentage (252.10%). Malik *et al.* (2000) observed maximum juice when zinc sulphate was applied on the mandarin trees. Li *et al.* (2007) stated that foliar application of calcium carbonate increased the juice contents in mandarin fruits.

***Fruit diameter (mm)*:** The maximum value of fruit diameter (78.26 mm) was observed in T_4 application of $ZnSO_4$@ 4% followed T_1 (65.30 mm), T_3 (64.13 mm) and T_2 showed fruit diameter (58.53 mm) (Fig. 1c). But the treatments (T_4, T_3, T_2 and $T_{1)}$ were non-significant with each other. Untreated fruits exhibited minimum fruit diameter (46.23 mm). Fruit size and fruit weight was significantly increased by foliar application of zinc sulphate (Tariq *et al.*, 2007). Cerklewski (2005) observed a significant increase in fruit diameter of Balady mandarin and grapefruit by application of calcium carbonate.

***Total soluble solids (TSS, %)*:** Minimum level of TSS was recorded in control (T_0), 107.67%. Maximum total soluble

solids were obtained in T_4 ($ZnSO_4$ @ 4%) 12.86% followed by T_3 ($CaCO_3$ @ 3%) 12.13% and T_2 (11.6%). Untreated fruits exhibited the lowest total soluble solids as compare to treated fruits (Fig. 1d). Tixier *et al.* (2010) reported that foliar application of zinc sulphate increased the TSS in the fruits of mandarin. Shah *et al.* (2002) reported that foliar spray of calcium carbonate on Kinnow plants improved the TSS in mango, peaches and citrus as compared to those plants which were not sprayed (control).

***Ascorbic acid (mg 100 g^{-1})*:** Maximum level (92.464 mg 100 g^{-1}) of ascorbic acid contents were found in T_4 treatment followed by T_3 (70.46 mg 100 g^{-1}), T_2 (64.51 mg 100 g^{-1}) and T_1 (60.20 mg 100 g^{-1}) (Fig. 1e). Minimum ascorbic acid was found in untreated fruits (32.25 mg 100 g^{-1}). El-Mwnshawi *et al.* (1997) concluded that Zn spray increased ascorbic acid in Balady mandarin fruits. Elmar *et al.* (2007) reported that calcium increased the ascorbic acid contents thus retained fruit firmness and reduced storage breakdown in apple.

***Total phenolic contents (µg ml^{-1} FW)*:** The results revealed that T_4 showed maximum (85.56 µg ml^{-1} FW) total phenolic contents followed by T_3 and T_2 (78.65 and 64.10 µg ml^{-1} FW, respectively). While T_0 showed the minimum TPC 31.69 µg

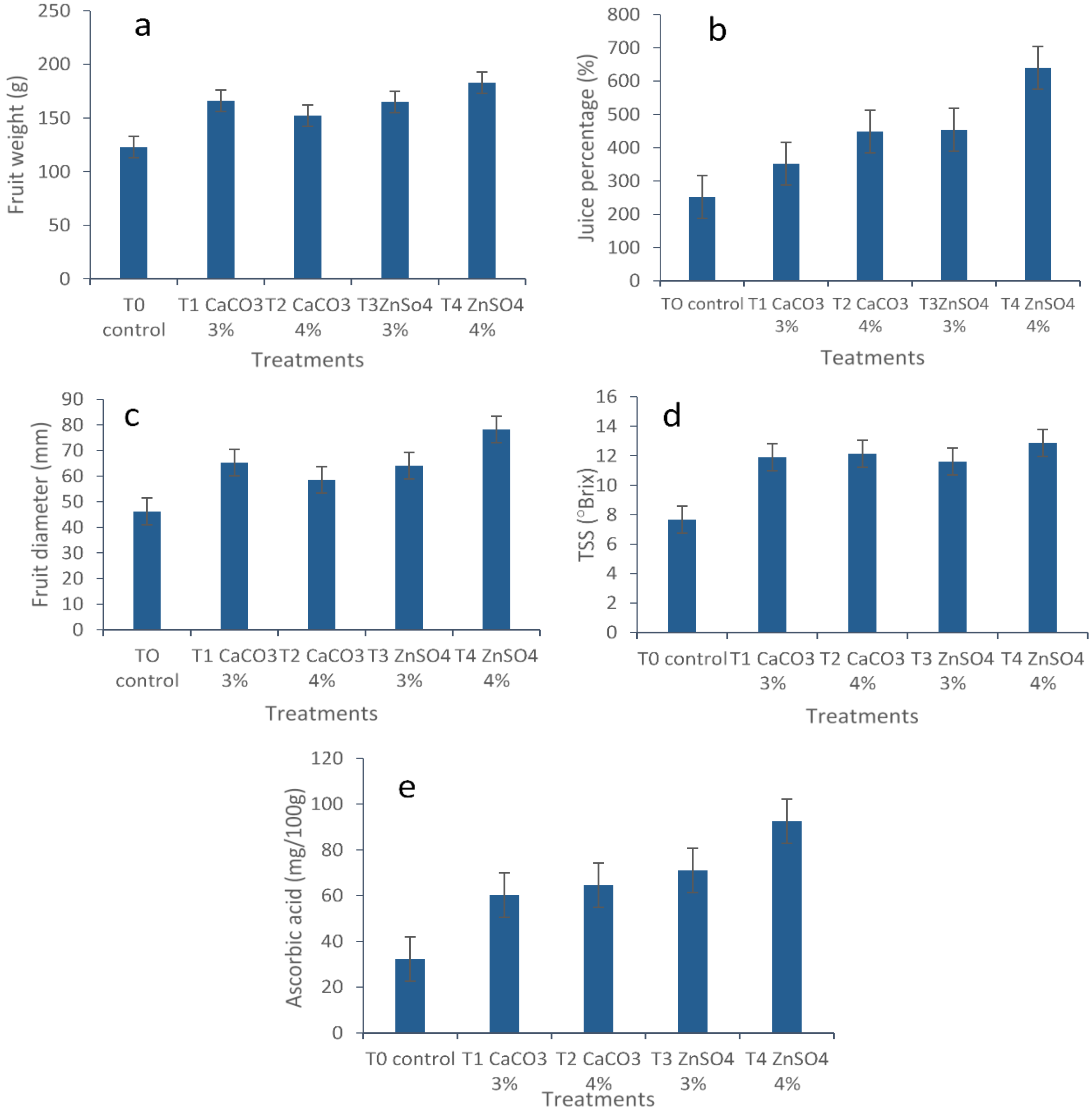

Figure 1. Effect of foliar application of zinc sulphate and calcium carbonate on fruit weight, juice percentage, fruit diameter, total soluble solids and ascorbic acid in Kinnow mandarin.

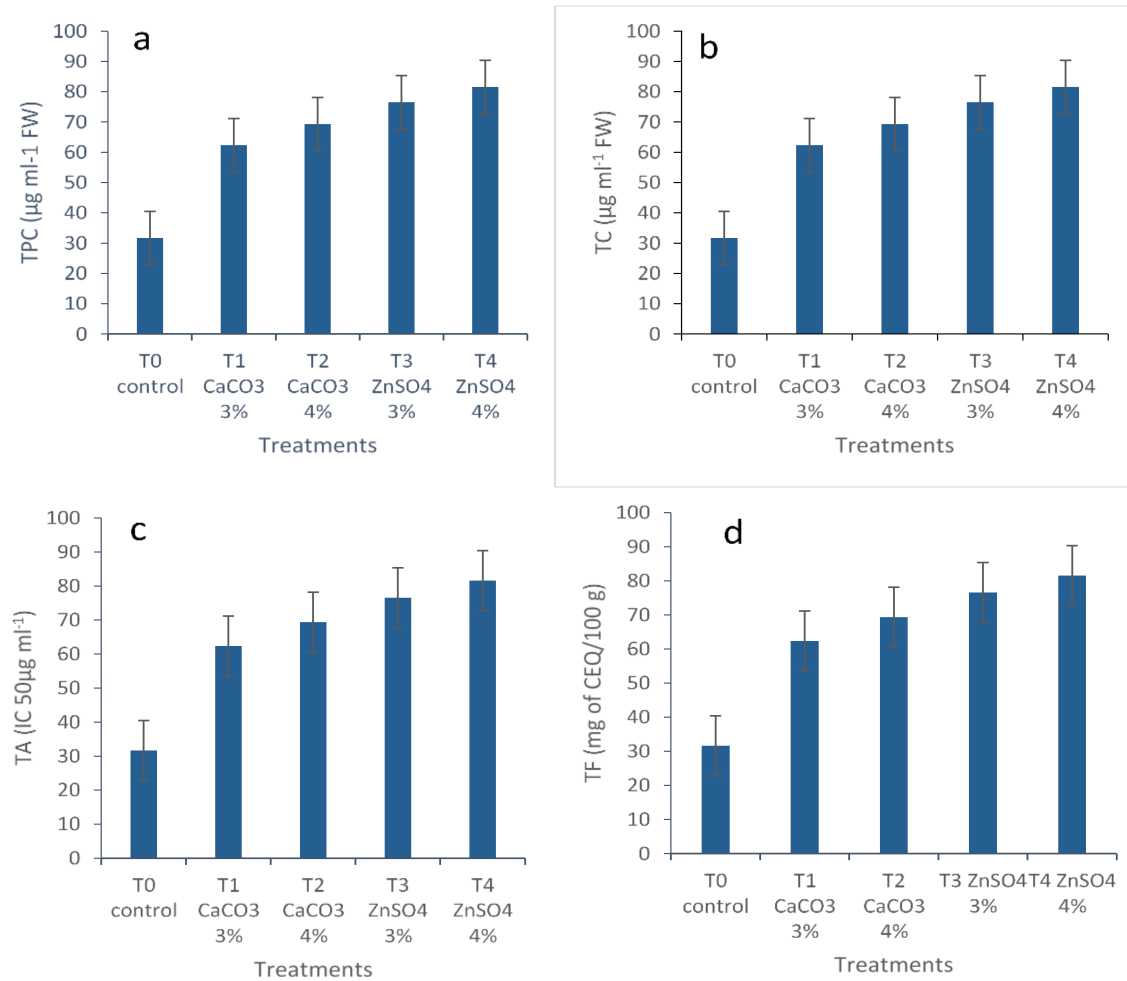

Figure 2. **Effect of foliar application of zinc sulphate and calcium carbonate on total phenolic contents, total antioxidants, total carotenoids and total phenolic contents in Kinnow mandarin.**

ml^{-1} FW (Fig. 2a). Phenolics present in fruits have been received considerable attention because of their potential antioxidant activity. Therefore, it can be concluded that the application of Zn and Ca increase the phenolic in Kinnow mandarin as compared to control. Cicco *et al.* (2007) reported that application of zinc sulphate increased the phenolic contents in Kinnow mandarin, lemon and grapefruits.

Total carotenoid contents (µg ml^{-1}FW)**:** Statistical data regarding total carotenoid contents of Kinnow fruit showed that maximum contents of total carotenoid (16.14 µg ml^{-1} FW) were observed by fruits of T_4 ($ZnSO_4$@ 4%) while untreated fruits showed minimum contents (11.89 µg ml^{-1} FW) of total carotenoids (Fig. 2b). Fruits of T_2 ($CaCO_3$@ 4%) and T_3 ($ZnSO_4$@ 3%) approximately showed the same value (14.12 and 15.76 µg ml^{-1} FW, respectively). Rodrigo and Zacarias (2010) reported that zinc sulphate @4-6% concentration somewhat increased the contents of carotenoids in *C. sinensis* and *C. reticulata.* Babu and Yadav (2002) proved that application of calcium carbonate appeared to increase carotenoids in peel of apple.

Total antioxidants (IC 50µg ml^{-1})**:** It is clear from the data that maximum total antioxidant (30.16 IC 50µg ml^{-1}) was observed in T_4 ($ZnSO_4$@ 4%) fruits followed by T_1 ($CaCO_3$@ 3%), T_3 ($ZnSO_4$@ 3%) and T_2 ($CaCO_3$@ 4%) (18.67, 26.52 and 20.45 IC 50µg ml^{-1}, respectively), as shown in Figure 2c. Berhow (2000) said that zinc sulphate increased the total antioxidant in Kinnow, grapefruit and lemons.

Total flavonoid contents (mg of CEQ/100 g)**:** It is obvious from the results that application of T_4 ($ZnSO_4$@ 4%) and T_3 ($ZnSO_4$@ 4%) showed maximum contents (46.43 mg of CEQ/100 g) and (44.97 mg of CEQ/100 g, respectively) of total flavonoid in comparison to fruits of control (T_0) which was 40.25 mg of CEQ/100 g (Fig. 2d). Fruits of both T_1 ($CaCO_3$@ 3%) and T_2 ($CaCO_3$@ 4%) received 42.43 and 43.06 mg of CEQ/100 g, respectively, of total flavonoid contents. Awad and Jager (2002) reported that zinc sulphate

treatments considerably increased the total flavonoid contents in apple, plum, peaches and citrus.

***Conclusion*:** The variation in quality attributes observed in this study concludes that preharvest foliar spray of zinc sulphate and calcium carbonate induced a reasonable change in Kinnow fruits. All physical and chemical characteristics improved with spray of both zinc sulphate and calcium carbonate. It is, therefore, suggested to adopt foliar application of zinc sulphate and calcium carbonate as preharvest spray.

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